REMARKS

Upon entry of the foregoing amendment, claims 1-13, 15-21, 26-31 and 42 are pending. Claims 1 and 31 have been amended. New claim 42 has been added. Claims 14, 22-25 have been cancelled, and claims 32-41 have been cancelled as being drawn to a non-elected invention.

No new matter has been added to the application. Any amendments to and/or cancellation of the claims was done solely for the purpose of expediting prosecution of the present application. Support for the amendment to claim 31 can be found in the specification as filed, for example at page 20, lines 30-32. Support for new claim 42 can be found in the specification as filed, for example at page 4, lines 7-12. Support for new claim 43 can be found in the specification as filed, for example at page 19, lines 21-24. Support for new claim 43 can be found in the specification as filed, for example at page 20, lines 30-32. Applicants reserve the right to pursue the subject matter of the claims as originally filed in this or a separate application(s).

Claim Objections

The Examiner has objected to claims 1-31 for the following minor informalities: "the fourth line of base claim 1 begins with 'A mixture of two probes…' (and) the claims should be a continuous sentence without uppercase headings." (Office Action, p.2). The Examiner also points out that "the probes are referred to as 'Probe A' or 'Probe b' in most instances in the claims, which is in consistent with their designation as 'probe A and probe B' in line 4 of claim 1." (Office Action, p.2). Applicants have amended the claims and respectfully request that the foregoing objections be withdrawn.

Rejection of Claim 31 Under 35 U.S.C. §112

The Examiner has rejected claim 31 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. (Office Action, p.2). In particular, the Examiner points out that claim 31 "recites the limitation 'wherein the target sequence in a forensic technique…' which is indefinite since there is no active step in the claim."

Applicants have amended present claim 31 to recite that the target sequence is detected in a forensic technique such as prenatal screening, paternity testing, identity confirmation or crime investigation. Reconsideration and withdrawal of the rejection is respectfully requested.

Rejection of Claims 1-10. 18, 29 and 30 Under 35 U.S.C. §102

The Examiner has rejected claims 1-10, 18, 29 and 30 under 35 U.S.C. §102(b) as being anticipated by Yang *et al.* (Anal. Biochem. (1998) 259:272-274, listed on IDS of 9/15/2006). Applicants respectfully traverse this rejection n the grounds that Yang *et al.* fail to teach or suggest each and every element of the claimed invention. Reconsideration and withdrawal of the rejection in light of the following discussion is respectfully requested.

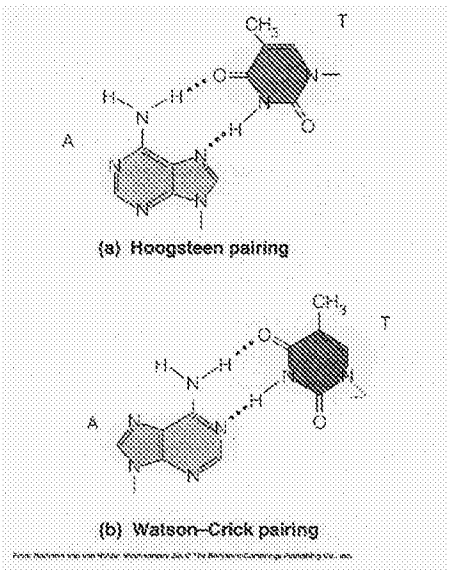
For a prior art reference to anticipate a claimed invention, the prior art reference must teach each and every element of the claimed invention. *Lewmar Marine v. Barient 827 F.2d 744, 3 USPQ2d 1766* (Fed. Cir. 1987).

Claim 1, as amended, is directed to a method for the analysis of a target sequence in a first sample, said method comprising contacting the first sample with a mixture comprising a mixture of probe A and probe B, wherein Probe A and Probe B are PNA probes, and wherein i) Probe A is directed to a region of the target sequence and is labeled with a fluorophore at the end which, upon hybridization is closest to the adjacent target region for Probe B; and ii) Probe B cohybridizes to a region of the target sequence adjacent to the target region of Probe A and is labeled with a quencher which, upon hybridization is closest to the adjacent target region for Probe A, and measuring fluorescence following cohybridization of Probe A and Probe B to the target sequence, under suitable hybridization conditions, wherein the presence or amount of target sequence present in the first sample can be negatively correlated with the fluorescence of the fluorophore on Probe A.

Yang *et al.* fail to teach or suggest a method as claimed, wherein ***Probe A and Probe B are PNA probes***. Accordingly, Yang *et al.* fail to anticipate claims 1-10, 18, 29 and 30.

The Yang *et al.* reference is directed to a DNA assay based on fluorescence resonance energy transfer (FRET) and DNA triplex formation. The specificity of the solution-based method taught by Yang *et al.* is based on **triplex formation, i.e. binding of probes to double-stranded target, and not duplex formation by binding of probes to single-stranded target,** as taught in the present reference. The method of targeting the wild-type homopurine sequences

I and II in p53 taught by Yang *et al.* is by two oligonucleotide probes that bind to their targets through Hoogsteen base-pairing (see, e.g. p. 272, first paragraph), and not Watson-Crick base pairing required by the present invention. Hoogsteen base pairs have different properties from Watson-Crick base pairs. The Hoogsteen geometry is the most favorable one for A-T base-pairs in solution, but not in double helices. G-C base pairs do not form Hoogsteen base pairs in solution, they are stable only in mildly acidic (pH4 - pH5) solutions when the N3 atom of cytosine is protonated and can participate in a hydrogen bond with the N7 of guanine. Hoogsteen G-C base pairs have only two hydrogen bonds, therefore, protonation is essential for pairing. The third Watson-Crick hydrogen bond ensures that the Watson-Crick pairing scheme is the most favorable in solution. A comparison is shown below.



In contrast, the present invention is directed to a method for the analysis of a target sequence in a sample using Probe A and Probe B that are PNA probes. As taught by the present disclosure, PNA is a non-naturally occurring polyamide that can hybridize to nucleic acid (DNA and RNA) with sequence specificity **according to Watson-Crick base pairing rules**. (see e.g., page 2, lines 4-8 of the specification). The binding properties of PNA provide novel characteristic of the PNA probes, as taught at pages 3-4 of the specification, for example:

> Higher Sensitivity: PNA binds stronger and faster to complementary RNA or DNA hereby facilitating the development of more rapid diagnostic tests. Where DNA probes often require over-night incubation, reactions with PNA are completed within a few hours (for example see: Thisted et al., Cell Vision 3:358-363 (1996)).
> Higher Specificity: PNA probes are particularly well suited for the discrimination between closely related sequences, even single nucleotide differences. This makes PNA-based assays highly specific (for example see: Igleo, G. L. BioTechniques 27:798-808 (1999)).
> Robust Assays: PNA is a synthetic molecule resistant to nucleases and proteases and thus extremely stable in prepackaged kit formats as well as during the actual assay where in contact with the sample (for example see: Demidov, Biochem. Pharmacol. 48:1310-1313 (1994)).
> Novel Assay Formats: The unique properties of PNA enable the development of assay formats, which go above and beyond the possibilities of DNA probes, hereby reducing the complexity related to the performance of molecular diagnostic tests (for examples see: Stender et al., J. Microbiol. Methods 48:1-17 (2002)).
> No Target Limitation: The non-charged backbone allows PNA probes to hybridize under conditions that are destabilizing to DNA and RNA. Attributes that enable PNA probes to access targets, such as highly structured rRNA and double stranded DNA, known to be inaccessible to DNA probes (See: Stephano & Hyldig-Nielsen, IBC Library Series Publication #948. International Business Communication, Southborough, Mass., pp. 19-37 (1997)).
> In Situ Hybridization: The hydrophobic nature of PNA relative to DNA makes PNA probes superior for in situ hybridization assays, where the probes must penetrate the hydrophobic cell wall prior to hybridization (for example see Stender et al., Int. J. Tuberc. Lung. Dis. 3:830-837 (1999)).

Clearly, the probes taught by Yang *et al.* are physically and functionally different from the PNA probes used in the methods of the present invention.

In view of the foregoing, Applicants respectfully submit that, contrary to the Examiner's assertions, Yang *et al.* fail to teach or suggest each and every element of the claimed invention and, thus, Yang *et al.* fail to anticipate the claimed invention. For the foregoing reasons, rejection of the claimed invention is believed to be improper and Applicants respectfully request that it be reconsidered and withdrawn.

Rejection of Claim 11 Under 35 U.S.C. §103(a)

The Examiner has rejected claim 11 under 35 U.S.C. §103(a) as allegedly being unpatentable over Yang *et al.* (as above) in view of Johansen *et al.* (U.S. Patent No. 6,441,152). In particular, the Examiner is of the opinion that:

> Yang teaches the limitations of claim 1-10, 18, 29 and 30…(h)owever Yang does not disclose methods wherein the PCR reaction is an asymmetric PCR reaction.
>
> Johansen teaches methods for detecting nucleic acid targets after binding to matrices containing non-nucleotide probes such as PNA probes…(and) Johansen further teaches that the amplification may be asymmetric PCR to favor production of a significant excess of single-stranded nucleic acid.
>
> It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Yang and Johansen since Johansen teaches a method for amplification of target nucleic acids that is highly compatible with the FRET probes taught by Yang for detection of mutant and wild-type target sequences. Thus, an ordinary practitioner would have been motivated to use the methods for asymmetric PCR in preparation of target nucleic acids which can then be detected in a closed tube assay using the FRET probes of Yang.

Applicants respectfully traverse this rejection. None of the cited references, taken alone or together, teaches or suggests the present invention as claimed. The present invention has been set forth above. As taught in the present application, for example at page 2, beginning at line 4 to page 4, line 17, there are many differences between PNA probes and standard nucleic acid probes, including biological, structural, and physico-chemical differences. For example, the hydrophobic character of PNA allows for the possibility of non-specific (hydrophobic/hydrophobic interactions) interactions not observed with nucleic acids. Further, PNA is achiral, providing it with the capability of adopting structural conformations the equivalent of which do not exist in the RNA/DNA realm, and result in a PNA polymer that is highly organized in solution. The considerable physico/chemical differences between PNA and DNA or RNA are described by Applicants at page 3-4 of the specification, as set forth above.

Further, Applicant's teach that the PNA probes functioning via FRET when hybridized to a target sequence, as taught by the present invention, were not contemplated by the prior art. For example, Applicant's teach at page 9, lines 21-32 of the specification that the presently claimed method using PNA probes A and B was not taught or suggested by the prior art:

> The use of PNA probe mixtures have previously been described as a way to target two adjacent target sequences (US2002058278), however, that concept required each of the two PNA probes hybridizing the adjacent target sequences to be extended with an arm segment capable of forming a triplex ***with a third labeled***

> ***PNA probe***, such that a total of three probes were required. ***The use of FRET was neither discussed nor proposed as a way to eliminate the need for "arms" and to avoid the complexity related to a third PNA probe. In fact, the use of a fourth probe or antibody was proposed***. (emphasis added).

As discussed above, the Yang *et al.* reference does not teach or suggest the claimed method, wherein Probe A and Probe B are PNA probes. The specificity of the solution-based method taught by Yang *et al.* is based on **triplex formation, i.e. binding of probes to double-stranded target, and not duplex formation by binding of probes to single-stranded target,** as taught in the present reference. The method of targeting the wild-type homopurine sequences I and II in p53 taught by Yang *et al.* is by two oligonucleotide probes that bind to their targets through Hoogsteen base-pairing, and not Watson-Crick base pairing required by the present invention.

The Johansen *et al.* reference is directed to methods, kits and compositions suitable for the detection of nucleic acids which are electrostatically immobilized to matrices using non-nucleotide probes, where the non-nucleotide probes which sequence specifically hybridize to one or more target sequences of the nucleic acid but do not otherwise substantially interact with the matrix, form a detectable complex. The Johansen *et al.* reference does not cure the defects of the Yang *et al.* reference. One of ordinary skill in the art would not look to the Johansen *et al.* reference and arrive at the method as claimed, wherein Probe A and Probe B are PNA probes. Neither Johansen nor Yang, taken alone or in combination, teaches or suggests the invention as presently claimed.

Accordingly, Applicants submit that the present claims are not obvious over the cited art. Applicants respectfully request that the foregoing rejection be withdrawn.

Rejection of Claims 12-17, 19-28 and 31 Under 35 U.S.C. §103(a)

The Examiner has rejected claims 12-17, 19-28 and 31 under 35 U.S.C. §103(a) as allegedly being unpatentable over Yang *et al.* (as above) in view of Livak *et al.* (U.S. Patent Pub. No. 2005/0053979). In particular, the Examiner is of the opinion that:

> Yang teaches the limitations of claim 1-10, 18, 29 and 30…(h)owever Yang does not disclose methods wherein the target or probes are immobilized to a surface such as an array or wherein one or both probes are blocking probes or linear beacon probes and/or comprise PNA or LNA.

> Livak teaches methods for design and making of oligonucleotide probes comprising universal base analogues for use in nucleic acid hybridization assays, and may also comprise other oligomeric structures such as locked nucleic acids (LNA) and peptide nucleic acids (PNA)…
>
> It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Yang and Livak since both references teach methods of nucleic acid hybridization using labeled probes, and measuring hybridization either by negative (Yang) or positive(Livak) changes in fluorescence of the donor moiety of a pair of FRET probes.

Applicants respectfully traverse this rejection. None of the cited references, taken alone or together, teaches or suggests the present invention as claimed. The present invention has been set forth above.

As discussed, the Yang *et al.* reference does not teach or suggest the claimed method, wherein Probe A and Probe B are PNA probes.

The Livak *et al.* reference is directed to nucleobase oligomers comprising universal base analogues that can be used, for example, in hybridization-based applications, including use as probes and primers. In certain examples, PNA or LNA structures are used to form the chain of specificity-determining and universal bases in the insulating combinatorial oligomer. The Examiner argues at page 10 of the Office Action that Livak *et al.* teaches "(e)nergy transfer probes can be provided as pairs of probes with donor and acceptor fluorophore moieties attaches to ends of the same probe or on separate probes, wherein changes in a detectable signal from the donor or acceptor moieties result from hybridization, usually as an **increase in measurable fluorescence**." (Emphasis added). Applicants point out that the present invention provides **negative correlation** between signal and the amount of target. Moreover, according to the present invention, fluorescence quenching occurs upon hybridization of the PNA Probes to their target sequences in contrast to other self-reporting PNA constructs previously described where fluorescence quenching occurs in the non-hybridized state. This is a fundamental difference that offers considerable performance advantages, such as higher specificity and/or sensitivity as will be discussed below leading to more reliable and robust assay formats. The difference between hybridization probes and PNA probes, is taught in the specification, for example at page 28, lines 8-26:

> Hybridization Probes and PNA Kissing Probes differs fundamentally even if Hybridization Probes where made of PNA. Hybridization Probes provide positive correlation between the signal and the amount of target whereas PNA Kissing Probes provide negative correlation between signal and the amount of target. A difference that is due to the fact that the measurable signal from Hybridization Probes comes from the emission of the acceptor fluorophore whereas the measurable signal from the PNA Kissing Probes comes from the emission of the donor fluorophore. Because of this difference, it is the donor moiety that is labeled with independently detectable fluorophore when two of more sets of PNA Kissing Probes are applied for multiplex assays as described in more details below, whereas in case of Hybridization Probes it is the acceptor fluorophores that differ. A difference that offers greater flexibility in the selection of fluorophore. PNA Kissing Probes are therefore not dependent on the emission from the acceptor moiety which allow the use of standard filter sets for the fluorophores whereas the Hybridization Probes need a combination of the excitation filter for the donor fluorophore and emission filter for the acceptor fluorophore. The use of Hybridization Probes is therefore limited to selected donor-acceptor pairs and instrumentation, such as the LightCycler, and not directly applicable for conventional fluorophore and instrumentation.

The Livak *et al.* reference does not cure the defects of the Yang *et al.* reference. One of ordinary skill in the art would not look to the Livak *et al.* reference and arrive at the method as claimed, wherein Probe A and Probe B are PNA probes. Neither Livak nor Yang, taken alone or in combination, teaches or suggests the invention as presently claimed.

Accordingly, Applicants submit that the present claims are not obvious over the cited art. Applicants respectfully request that the foregoing rejection be withdrawn.

CONCLUSION

In view of the above amendment, applicant believes the pending application is in condition for allowance. If a telephone conversation with Applicants' attorney would expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 449-6500.

Please charge any underpayments or credit any overpayments to our Deposit Account No. 50-4876, under Order No. 119880-00602 from which the undersigned is authorized to draw.

Dated: April 14, 2011

Respectfully submitted,

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